原　　著

Experimental Studies on Brephoplastic Transplantation in Mice

by

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INTRODUCTION

Several reports have appeared on brephoplastic transplantation[23)24)], which can be divided into three main categories according to their approaches. One has dealt with the immunological and theoretical basis of transplantation in general. Another has used brephoplastic grafts in the experimental pathology and embryology connected with cancer research, since embryonic tissue cells still possess a development potential and a viability which resembles the autonomy of cancer cells. The third group of reports has considered the question of whether brephoplastic grafts can be used clinically.

In all three categories, tissues from the very early embryonic stage have been used for brephoplastic grafts and have been transplanted to the brain[41)], the anterior chamber of the eye[5)], and the cheek pouch of the hamster, which have been recognized as favorable sites for graft survival. From the viewpoint of practical possibilities, the brain and the eye are not smooth sites that lend themselves to clinical application in humans.

The author therefore selected various tissues taken from mouse embryos in the last quarter of gestation for grafts and several regions in adult mice for transplantation sites. The author then investigated the biological behavior of brephoplastic transplantations in an attempt to clarify the following points.

(1) When embryonic tissues are transplanted heterotopically, what are the differences between isografts and homografts? (i.e., what is the antigenic potency of embryonic tissues?)

(2) What sites are favorable for brephoplastic grafts in addition to those already known, and what are the clinical applications?

(3) GREENE[15)16)18)] has shown that embryonic and malignant tumor tissues have in common the property of autonomy. When such viable tissues grow and develop indifinitely in unnatural surroundings, what is the fate of the surviving graft? Furthermore, does a constitutional abnormality in the host influence the brephoplastic graft? Can embryonal rests become malignant?

MATERIALS AND METHODS

Mice of pure strains C3H/HeMs ($H\text{-}2^k$)[35)] and A/Jax ($H\text{-}2^a$)[35)] supplied by the Animal Center of Kyoto University were most frequently used for both grafts and hosts,

but some C58(H-2^K)[35] and dds mice were also used.

Three age groups were made; 14-15, 16-17, 18-19 day embryos. The age of the embryo dated from the time a pregnant female mouse was separated from an inbred male mouse after coitus for 48 hours. Under sterile conditions, embryos taken from female mice on the stated day of gestation was washed thoroughly in physiologic saline solution at approximately 15°C to remove antigenic substances of the mother's body. Then the embryo was dissected and divided into various kinds of tissues, which were minced into less than 2×2 mm pieces. These small fragments were placed in an injection needle with an internal diameter of 2 mm and were transplanted almost intact into several sites in adult host mice.

Newborns within 12 hours after birth were occasionally used as sources of grafts. The recipient hosts were two month old mice weighing 20 to 25 g. In this experiment, the sex of the host did not matter. Most hosts were not treated. Some were given subcutaneous injection of cortisone-acetate (0.02 mg/g of body weight) on alternate days from the time of transplantation until the 14th postoperative day.

Transplantation combinations were as follows:

(A) Sources of embryonic graft tissues;

1. ectodermal origin : skin } limb bud
2. mesodermal : cartilage or bone } limb bud
3. endodermal : stomach, intestine, liver, lung, heart and kidney } minced whole tissues
4. placenta

"Minced whole tissues" represent semi-fluid substances consisting of various tissues probably originating from all three germ layers, which were prepared by mincing and slicing the whole body except for the head. Approximately 0.1 cc was used for grafting.

(B) Transplantation sites in adult hosts;

1. subcutaneous space of abdominal or anterior chest wall
2. intramuscular space of thigh muscle
3. retrorenal space on the left side
4. infrequently, chest cavity and uterine lumen

In transplanting into the retrorenal space or uterine lumen, all grafts were inserted by open operation, so that accurate transplantation into site desired would be possible.

(C) Combinations of respective mouse strains, grafts and transplantation sites;

1. isologously, for example, 16-17 day embryonic skin of A/Jax was transplanted into the thigh muscle of adult of the same strain.
2. homologously, for example, 14-15 day minced whole tissues of A/Jax were, by open operation, inserted into the retrorenal spaces of cortisone-conditioned C3H mice.

All hosts were examined for graft survival. Animals were sacrified at 2, 7, 14, 21, 28, 30 days and longer postoperatively. Grafts containing the transplanted beds were fixed in 10% formalin and stained with hematoxylin-eosin or sometimes van Gieson's for histological studies.

Observations of homografts were completed by two months, but some isografts were followed throughout the life-span of the host.

RESULTS

Section (A) The biological differences between isologous brephoplastic grafts under several conditions.

Table 1 records the results when various tissues of embryos older than 14-15 days were transplanted to various regions of adult mice. The term 'take' is used to signify an observed increase in size and the healthy state of the graft, in which the structures persist without being destroyed.

The grafts were sometimes cortisone-treated by the methods described above.

The data recorded in this Table lead to the following conclusions:

Table 1. The transplantabilities of heterotopic brephoplastic grafts in mice under several conditions.

Sources of embryonic grafts		Transplantation sites in adult mice			
		Subcutis	Intramuscular	Retrorenal	Other sites
type of tissues	ages in days	takes no. /total no. strain	takes no. /total no. strain	takes no. /total no. strain	takes no. /total no. strain
Skin	14-15	5/7 dd	13/14 A/Jax		
			# 3/10 A/Jax-C3H		
	16-17	6/10 A/Jax	30/34 A/Jax	5/8 A/Jax	
			# 4/13 A/Jax-C3H	# 3/8 A/Jax-C58	
	18-19		9/12 A/Jax		
			# 3/13 A/Jax-C3H		
	newborn	1/4 A/Jax	5/8 A/Jax		
Cartilage or bone	14-15		# 6/13 A/Jax-C58		
	16-17		3/5 A/Jax		
			# 2/7 A/Jax-dd		
Limb bud	14-15		# 5/13 A/Jax-C58		
	16-17		4/7 A/Jax	5/7 A/Jax	
			# 2/7 A/Jax-dd	# 2/8 A/Jax-C58	
Stomach	18-19		9/13 A/Jax		0/7 C3H (chest cavity)
			7/9 C3H		
			# 4/10 A/Jax-C3H§		
	newborn	1/4 A/Jax	3/4 A/Jax		
		# 1/10 A/Jax-dd			
Intestine	14-15	2/7 dd	4/5 C3H		
	18-19	1/6 A/Jax	11/16 A/Jax		0/7 dd (chest cavity)
		# 0/4 A/Jax-dd	# 3/11 A/Jax-C3H		
	newborn	1/8 A/Jax	3/4 A/Jax		
Lung	14-15		# 0/5 C3H-A/Jax		
	16-17		2/5 A/Jax		
			0/8 dd		
	18-19	0/6 A/Jax	2/6 A/Jax		
			3/10 C3H		

	newborn	0/4 A/Jax	0/6 dd ♯ 0/11 A/Jax-C3H ♯ 0/6 A/Jax-dd 2/6 A/Jax 0/4 C3H		
Heart	14-15 16-17		0/7 dd 0/5 dd		
Liver	14-15 16-17 18-19 newborn		0/5 C3H ♯ 0/8 C3H-A/Jax 0/6 C3H 0/5 C3H 0/6 C3H		♯ 0/12 hamster (cheek pouch)
Kidney	14-15 18-19 newborn		0/5 C3H ♯ 0/8 C3H-A/Jax 0/10 C3H 0/6 C3H 0/6 A/Jax		
Minced whole tissues	14-15	2/6 C3H ♯ 0/6 C3H-A/Jax	3/6 C3H ♯ 3/7 C3H-A/Jax§	5/6 A/Jax ♯ 9/20 A/Jax-C58§ ♯ 5/12 A/Jax-C3H§	
	16-17	2/7 C3H	4/7 C3H	7/7 A/Jax 4/5 C3H 8/8 C58 ♯ 2/4 A/Jax-C3H ♯ 6/10 C3H-A/Jax	0/5 C3H (uterine) 0/7 C58 (uterine)
	18-19			6/8 A/Jax	
Placenta	16-17	0/9 dd			

- : For example, 'A/Jax-C3H' means that embryonic tissues of A/Jax were transplanted in adult mice of C3H strain.
♯ : Homologous brephoplastic transplantation.
§ : Recipient hosts cortisone-treated.

(1) Generally, heterotopic brephoplastic isografts survived and developed well. A high percentage of skin, stomach, intestine, cartilage and limb bud tissues transplanted to intramuscular spaces survived and grew. Minced whole tissues were also successful isografts in either the intramuscular or the retrorenal spaces.

(2) On the contrary, homologous intramuscular grafts of skin, stomach, intestine and cartilage survived in only a few cases for 5 weeks. They became surrounded by cell-infiltration and were usually rejected by 5 weeks after the transplantation.

(3) Isografts of lung tissue to intramuscular spaces occassionally survived, but liver, heart, kidney (also of endodermal origin) and placenta transplants never took.

(4) The percentage of successful takes did not vary with the age of the embryos.

(5) The intramuscular and retrorenal spaces were more favorable sites for brephoplastic transplantation than the subcutis.

Section (B) Differences in transplantability of skin and intestine of embryos of

different ages to the intramuscular space and the influence of cortisone administration to the hosts.

Since skin and gastroenteric tissues were recognized as the most successful brephoplastic grafts and both tissues tended to cause cyst formation in the thigh, as will be described in the next section, the author used these tissues exclusively as grafts and the intramuscular space as the site of transplantation, in a study of the percentage of take at different embryonic ages. The effect on the brephoplastic homograft of cortisone administration to the host was also investigated.

The results are shown in Table 2 and Fig. 1.

Table 2. Takes of intramuscular heterotopic grafts of skin and intestinal tissue of various ages of mouse embryos.

Age & graft of embryo	Isologous				homologous				
	Total no.	Takes	(Percent)	Strain	Total no.	Takes	(Percent)	Strains	Cortisone to hosts
14-15 day Skin	14	13	93(%)	A/Jax	6	2	33(%)	A/Jax-C3H	(+)
					10	3	30	A/Jax-C3H	(−)
					10	2	20	A/Jax-C58	(+)
					8	2	25	A/Jax-dd	(+)
16-17 day Skin	34	30	88	A/Jax	10	3	30	A/Jax-C3H	(+)
	8	4	50	C3H	13	4	31	A/Jax-C3H	(−)
18-19 day Skin	12	9	75	A/Jax	14	4	28	A/Jax-C3H	(+)
					13	4	31	A/Jax-C3H	(−)
					8	4	50	A/Jax-C58	(+)
					6	3	50	C3H-C58	(+)
Newborn Skin	8	5	62	A/Jax					
	10	2	20	C3H					
14-15 day Intestine	5	4	80(%)	C3H	16	4	25(%)	A/Jax-C3H	(+)
	8	5	63	dd	12	6	50	A/Jax-C3H	(−)
					6	2	33	C3H-A/Jax	(−)
					5	2	40	dd-C3H	(+)
16-17 day Intestine	12	10	83	A/Jax	10	4	40	A/Jax-C3H	(+)
	12	9	75	C3H	10	2	20	A/Jax-C3H	(−)
18-19 day Intestine	16	11	69	A/Jax	9	3	33	A/Jax-C3H	(+)
	♯ 9	7	78	C3H	11	3	27	A/Jax-C3H	(−)
					6	2	33	A/Jax-C58	(+)
Newborn Intestine	4	3	75	C3H					
	♯ 9	4	44	C3H					

(+), (−) : The recipient hosts cortisone-treated or not. ♯ : Graft of stomach tissue.

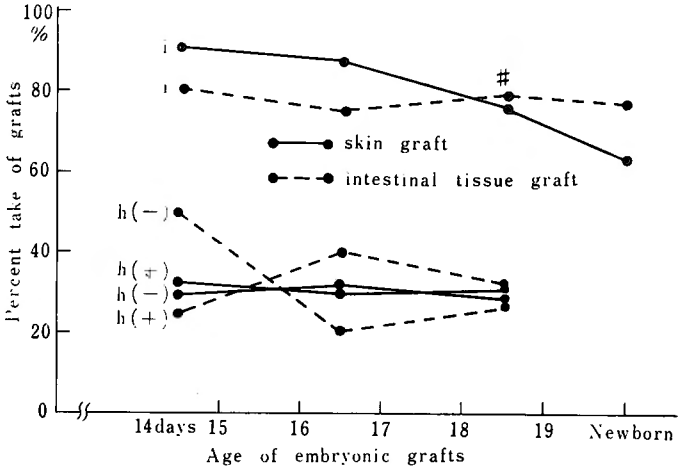

Fig. 1 Percentage take of intramuscular grafts of skin and intestinal tissue. Compare with Table 2. i: isograft, h: homograft, (+), (−): cortisone-treated hosts or non-treated, #: stomach graft.

(1) While isografts grew well, homografts failed in 50% of the cases.

(2) With advancing embryonic age, the number of skin and intestinal isografts decreased gradually. However, no difference in percentage of takes of homografts was found at various embryonic ages. (See the curves in Fig. 1)

(3) When brephoplastic grafts of both skin and intestine were transplanted into thigh muscles,either isologously or homologously, the percentage of takes was about equal.

(4) Cortisone administration seemed to have no effect on the length of survival of brephoplastic homografts[36)37)].

Section (C) The ultimate fate of brephoplastic grafts; gross and histomorphological appearance of long surviving grafts.

Long surviving isografts of skin and intestine were examined histologically at selected intervals as illustrated in Tables 3 and 4. Minced whole tissues implanted in the retrorenal spaces were observed for long periods. The others also were examined histologically, and the following interesting information on the behavior of brephoplastic grafts was obtained.

Table 3. Survival time of intramuscular isografts of skin.

Age of graft (days)	Total no.	No. surviving after transplantation				
		10 weeks	25 w.	50 w.	398 days	Takes
14–15	14 (C3H)	13				13
16–17	34 (A/Jax)	4	11	11	4	30
	8 (C3H)	4				4
18–19	12 (A/Jax)	7	2			9
Newborn	8 (A/Jax)	5				5
	10 (C3H)	2				2

Table 4. Survival time of intramuscular isografts of ♯ stomach and intestinal tissue.

Age of graft (days)	Total no.	No. surviving after transplantation				
		10 w.	20 w.	45 w.	366 d.	Takes
14-15	5 (C3H)	3	1			4
	8 (dd)	5				5
16-17	12 (A/Jax)	4		4	2	10
	12 (C3H)	6	3			9
18-19	♯ 13 (A/Jax)	5	4			9
	16 (A/Jax)	11				11
Newborn	♯ 4 (A/Jax)		3			3
	4 (C3H)	3				3

(1) Skin: Isografts of embryonic skin showed full growth in the muscles of adult mice. By about the 14th day, a cyst had developed from a fragment of skin which had been transplanted as a flat structure. At this time, the cysts measured $2 \times 7 \times 7$ mm on the average and were lined with stratified squamous epithelium containing hair follicles and sebaceous glands. Hairs actually projected into the cyst. By ten weeks, the cysts averaged 12×8 mm in size. They stopped growing and remained indefinitely in healthy state.

It is possible that these embryonic skin fragments formed 'dermoid cysts' with walls of skin including all its appendages, often with prominent sebaceous glands, as shown in Figs. 2, 3, 4 and 5. These walls gradually degenerated and finally lost their appendages (267 days after transplantation). The cysts were made up of stratified epidermis surrounded by fibrous tissues and contained keratinized debris. These may be called 'epidermoid cysts' (Figs. 6, 7 and 8).

Homologous skin grafts showed the same features as isologous in the earlier stages after transplantation. However, their development was much slower. The cysts were smaller than isologous ones at the corresponding period. Cellular infiltration developed around them between 3 and 4 weeks (Figs. 9, 10 and 11).

(2) Cartilage or bone and limb: When these grafts were transplanted isologously or homologously, they persisted indefinitely in some sites. However, they showed no vigorous growth. After one month, bone marrow, osteoid tissues and endochondral ossification appeared[8]. Many limb buds in the retrorenal spaces developed small digits of cartilage, stratified epithelium and connective tissues. (ref. Fig. 18)

(3) Stomach and intestine: As with skin grafts, stomach and intestinal grafts tended to produce cysts in the sites of transplantation. Isologous intestinal grafts grew rapidly and formed cystic tumors which could be recognized grossly by the 14th postoperative day or so. By about 5 weeks after transplantation, the tumors averaged 1.0×0.8 cm. These cysts contained clear fluid and sometimes burst into the adjacent tissues slightly (Fig. 17).

Histologically, they showed a concentric organization of secretory epithelium, lamina propria and unstriated muscle layer by 7-14 days. This arragement was similar to that

seen in adult organs. In the secretory epithelium covering the villi were numerous mucous cells and a few goblet cells with mitotic figures. Infiltration of host cells around the graft was scarce throughout the period of observation (Figs. 12, 13 and 14).

During the 20 week period of observation, the epithelium became degenerated and partly sloughed into the cystic lumen as debris. However, the unstriated muscle layers were well preserved for even longer periods. This finding suggests that differentiated muscle layers interfere with the blood supply from the host to the lamina propria of the graft.

Stomach grafts showed the same general organization as intestinal grafts.

The graft cells never infiltrated or invaded the adjacent tissues.

The homograft cysts were almost always smaller than the isografts. Histologically, cellular infiltration gradually surrounded the homograft between 2 and 3 weeks after transplantation, and the organization of the homograft was destroyed as the host's cellular infiltration increased (Figs. 15 and 16). The rejection reaction became progressively greater. In about two thirds of the cases, the cystic structures once formed were destroyed. The remaining grafts were more or less rejected by 5 weeks.

This behavior varied neither with the age of the embryo nor with the sex of the host. Intestinal homografts in cortisone-treated hosts had a similar fate.

(4) Lung: Although a few intramuscular isografts of lung tissue were taken at first, they were finally absorbed by 3 weeks or only a trace of fibrotic tissue remained. Histologically, the graft contained numerous respiratory epithelial cells or alveoli (Figs. 19 and 20). Some even developed dilated dilated alveoli, but these soon became compressed. Neither prominent enlargement nor development of bronchial buds was seen.

(5) Liver, heart and kidney: All liver grafts began to be absorbed from the moment of transplantation. Heart and kidney grafts always diminished within 7 days. It was very difficult for grafts of these tissues to take in any of the several sites attempted in this experiment.

(6) Minced whole tissues particularly in the retrorenal space: Isologous minced whole tissues inserted in the retrorenal space survived more often and for longer periods. There were a few successful homologous grafts. Three months after transplantation, the grafts had formed irregularly round masses approximately 1.2×1.8 cm in size (Fig. 21). The following organs and tissues were most frequently present in these masses, some of them shown in Figs. 22, 23, 24 and 25: skin with zoned epidermis and hair follicles, small areas of cartilage with endochondral ossification, bone marrows with bone trabeculae, gastroenteric tissues with cysts of mucous epithelium, lung tissues with respiratory epithelium and sometimes ganglia. There were various tissue components in one mass. Not infrequently, tissue components of two germ layers appeared. At times the mass consisted of components derived from all three layers. Such a histological complexity was strongly similar to that of spontaneously occurring mixed tumor or teratoma. (ref. Fig. 26)

(7) Placenta: Isologous 16-17 day old placentas were implanted subcutaneously in female mice. There was no growth, and all became necrotic within 7 days.

Section (D) Relationship between brephoplastic graft and tumor-bearing host in mice.

The aim of this experiment was to determine whether or not brephoplastic grafts acted abnormally or became malignant in tumor-bearing hosts.

The hosts were all female mice of the C3H strain. Skin and intestinal tissue of 16-17 day embryos were used for the grafts. A mammary adenocarcinoma, which arose spontaneously in a C3H female mouse, was excised, minced and made into a tumor cell suspension. Fixed volume of this suspension were injected subcutaneously into the backs of adult female mice of the same strain. Seven days later, embryonic skin or intestinal tissue was again transplanted to the thigh muscle of the host. The procedures described in Table 5 were carried out in the other groups, and the grafts were observed for 60 days.

Table 5. Relationship between brephoplastic grafts and tumor-bearing hosts.

Graft group / Host group	(1)	Skin No. takes/total no.	percent	(2)	Intestine No. takes/total no.	percent
A	A-1	6/13	46%	A-2	16/20	80%
	A-1′	6/28	21	A-2′	7/30	23
B	B-1	6/12	50	B-2	13/16	81
C	C-1	8/18	44	C-2	9/16	57
Control	See Table 2.					

Group A : Brephoplastic grafts of 16-17 day embryo transplanted into thigh muscle of host with tumor transplanted into the back.
Group B : Tumor and embryonic tissues transplanted simultaneously to two different sites in the same mouse.
Group C : Tumor transplanted 7 days after brephoplastic transplantation.
A-1′ and A-2′ : Brephoplastic homografts in the hosts of group A.

(1) Relationship between isograft and tumor-bearing host: In general, no difference in percentage of take was noted between the experimental and the control groups. The percentage of takes was higher in group A-2 than in group C-2. During the period of observation, the cysts in group A-2 reached the size of approximately 1.5×1.2 cm, which was larger than in any of the other groups (Fig. 27). Encystment in group C-2 was poor and weak.

There was, however, no evidence that embryonal rests differentiated abnormally or became malignant even in constitutionally abnormal hosts, and these grafts had the same appearance as those described in the previous section.

There was no direct parallel between the growth of the graft and the size of the tumor in the same individual.

(2) Relationship between homograft and tumor-bearing host: Skin and intestinal tissue from 16-17 day embryos of the A/Jax strain were transplanted to tumor-bearing hosts of the C3H strain, which had been prepared seven days previously by the methods mentioned above (groups A-1′ and A-2′).

Six of the 28 skin and 7 of the 30 intestinal homografts survived for 5 weeks. These results are very similar to the percentage of takes recorded in the former experiment.

DISCUSSION

Tissues, which are incompatible in the adult stage, are often compatible homografts when transplanted in the embryonic, fetal or neonatal stage, as GREENE[19] reported in 1955.

However, even if embryonic tissues are transplantable homografts, other factors of course participate in the mechanism of transplantation immunity when the brephoplastic transplantation is performed in strains with a difference in histocompatibility-2.

Antigenicity in the embryo has been believed to be very poor or absent. The ontogeny of transplantation antigens has recently been studied by several workers.[13)26)29)31)] Using accelerated skin graft rejection as a test of antigenicity, BILLINGHAM et al.[3)], and CHUTUNA and HASEK[7)] detected transplantation antigens in 11•1/2 day and 8 day old mouse embryos respectively. MÖLLER[25)] demonstrated antibody-absorbing antigens in embryos over 15 days old, and DORIA[12)] also found transplantation antigens in the hematopoietic tissue of mouse embryos of the same age. PIZARRO et al.,[29)] on the other hand, reported that antigen was first detectable, on the basis of absorption, in the livers of young mice about 3 days after birth. The disparity in these data on the development of transplantation antigens may be due to the differences in testing techniques, mouse strains and tissues used.

Now, if skin and intestinal tissues of mouse embryos over 14-15 days old have little or no antigenicity, homografts transplanted in adult mice, which have reached complete immunological maturity, should survive for a long time without causing antibody reaction in the host. The results described in Section (B) were contrary to this expectation. While isografts of embryonic skin and intestinal tissue survived and continued to grow permanently even in intramuscular spaces, homografts almost never survived for long. Moreover, homografts incited a typical host versus graft reaction similar to that seen in homotransplantations of adult normal organs and tissues. Homografts of intestinal tissue induced particularly intense rejection reactions. The take rate of homografts did not vary with the age of the embryo. These findings imply that transplantation antigens in skin and intestinal tissue of embryos older than 14-15 days have already developed to a considerable degree. The author's conclusions regarding the development of transplantation antigen, obtained from the heterotopic homograft capacity of embryonic tissues, agree in principle with the evidence presented by BILLINGHAM et al.,[3)] although the methods of detecting antigenicity differed in many respects.

It is noteworthy, however, that although there were variations according to strain, type of embryonic tissue and site of transplantation, homografts of embryonic tissues were apt to survive longer than the average survival time of homografts between adult mice. This characteristic of brephoplastic grafts suggests that the antigenic specificity and maturity of embryonic tissues have not yet attained adult levels.

In this experiment the author used two month old mice exclusively as hosts. If hosts were chosen at an age before their immunity response attacked the grafts, brephoplastic transplantations might be more successful. GROUSE[9)11)11)], for example, investigated the role of the host-donor age conbination, using embryonic stomach transplanted into rat brain. Watanabe[38)] obtained good results with mice when brain tissue taken from 11-13 day embryos was transplanted into the brains of 12-48 hours old hosts. CANNON et al.[6)] performed similar experiments with chick skin homografts, and concluded that the tissue specificity or antigenicity of chick skin is developing at the time of hatching and complete by the 14th day and that the abilty to resist homografted tissue is also developing in the chick at the time of hatching, but is absolute by the 7th day post-hatching.

The incomplete ability of embryonic tissue to elicit a homograft reaction means that there is little hope for the clinical use of brephoplastic transplantation[22]. For practical purposes, more favorable sites should be picked for brephoplastic graft survival than the brain or the eye. The results mentioned in Section (A) suggest that the intramuscular and the retrorenal spaces are somewhat suitable for brephoplastic grafts. The former is particularly easy to use.

The use of human embryonic, fetal or neonatal tissues to repair burned surfaces[20] and endocrine deficiencies has been reported by several investigators[28]. The results of these reports are encouraging enough to suggest the possibility of brephoplastic transplantation. GAILLARD[38], for example, reported the successful transplantation of parathyroid tissues taken from newborn or stillborn human fetuses to patients with hypothyroidism. Following transplantation, their tetany disappeared and calcium levels rose. BAXTER and GOLDSTEIN[14], who transplanted skin from 17 to 33 week old fetuses to adults, found that the survival time of the grafts ranged from 4 to 46 days.

Brephoplastic transplantation has many applications in cancer resarch. In GREENE's opinion,[15][16][18] embryonic and malignant tumor tissues have in common the property of autonomy. The developing state of the embryo, in other words, may be identical to 'promoting process' of cancer[4][27]. The concept that tumors arise from embryonic cells which have been lying latent and then are stimulated to grow again has been prominent in the history of cancer research. Human cases have made clear not only that the adult body now and again contains embryonal rests but that from them tumors may arise, often growths of extreme malignancy. Thus, it is important to observe the ultimate fate of long surviving embryonic grafts in unnatural environments.

The present experiment confirmed the finding that some embryonic tissues grew and differentiated vigorously in the heterotopics. Histomorphological examinations continuing for the majority of the host's life-span provided some interesting informations which had not hitherto been recorded.

Embryonic skin fragmenets surviving in thigh muscles produced 'dermoid cysts'[2] in an early stage, which then changed into 'epidermoid cysts'[2], the walls of which consisted of degenerated thin epithelium without skin appendages. 'Minced whole tissues' in the retrorenal spaces also developed and formed large round masses, in which several tissues originating from all three germ layers became well differentiated. The histological appearance was that of a mixed tumor or teratoma[2][34]. This experimental production of 'teratomas' from embryonal rests is very interesting in the light of clinical experiences that teratoid tumors arise not infrequently in the retroperitoneum.

However, long surviving grafts, including 'teratoma', showed nothing but normal differentiation as they developed. They never invaded neighboring tissues of either the hosts or the grafts themselves as malignant tumors do. The results of numerous studies in which embryonic material was transplanted to the brains or the eyes in mammals gave no evidence that malignancy can arise from embryonic tissues, even though they survived for a long time in these sites. WILLIS[39][40] stated that many of the tissues and organ rudiments grew and differentiated nearly normally when whole 6 mm rat embryos were transplanted into the brains of young rat hosts.

On the other hand, carcinogens acting embryonic tissues can often induce malignant

tumors in them. SMITH[32)33)] and ROUS[30)], in the first experiment of this kind, found that embryo skin with oil containing methylcholanthrene transplanted in adult mice soon caused tumors to develop. KLEIN[21)] succeeded in inducing tumors following the intrasplenic or intrahepatic transplantation of fetal skin, muscle or stomach with methylcholanthrene in mice. GREENE[1)17)] also studied the behavior of homografts and heterografts of embryonic rabbit and rat skin infected with Shope papilloma-virus.

An experiment was undertaken to determine whether or not constitutional abnormalities of the host have any influence on the growth of brephoplastic grafts. Tumor-bearing female mice were used as hosts. Although the results of this experiment did not answer this question completely, it is remarkable that embryonic intestinal tissue transplanted to hosts with tumors which had started one week before and were now increasing in size produced larger cystic tumors than those in any other group. Cyst formation was poorest in group C-2. It is, however, still not clear whether or not constitutionl abnormalities in tumor-bearing animals acelerate the growth of embryonic rudiments. If an abnormal constitution, such as that of cancer patients, increases the survival of brephoplastic grafts, it is possible that brephoplastic transplantation may have a therapeutic use in cancer patients, for example, embryonic bone marrow grafts could be used to treat anemia.

In the last experiment with the same materials (groups A-1' and A-2'), no significant difference was detected between brephoplastic homografts in abnormal and in normal hosts.

SUMMARY

Small fragments of various tissues taken from embryos older than 14-15 days were heterotopically transplanted to several sites of two month old adult mice isologously or homologously. Histological examination showed certain characteristics of brephoplastic transplantation. The following problems are considered: antigenic specificity and immunological development in the embryo, the clinical applications of brephoplastic grafts and the possibility of malignant changes in embryonal rests.

(1) In general, skin, gastroenteric tissues, cartilage or bone, limb bud and lung grew and differentiated well in the thigh muscles of hosts of the same strain. Skin grafts survived for as long as 398 days and intestinal grafts for 366 days. Skin fragments produced 'dermoid cysts' first, which changed into 'epidermoid cysts'. Intestinal grafts also formed fluid cysts, in the walls of which were seen general concentric arragements.

(2) Homologous intramuscular grafts of skin and intestinal tissue often survived for 21 to 28 days. Cellular infitration in the host began to attack the homograft two to three weeks after transplantation. The graft structure, which was first as healthy as that of isografts, was more or less destroyed by 5 weeks.

Limb buds persisted longer but did not develop well. There was bone marrow formation in only a few cases.

(3) Only a few isologous lung grafts in intramuscular spaces survived. Liver, heart and kidney tissues were unsuccessful in all sites.

(4) Minced whole tissues in the retrorenal space produced round tumors, in which various tissues differentiated normally to form 'mixed tumors' or 'teratoma'.

(5) The next experiment was designed to determine the difference in incidence of takes between isografts and homografts and the variation with embryonic age. Although

isografts of skin and intestinal tissues in intramuscular spaces survived indifinitely without host reaction, homografts were all rejected within 5 weeks by a typical rejection. These data indicate that antigenic specificity in skin and intestine of mouse embryos older than 14-15 days have already developed to a considerable degree.

(6) The intramuscular and retrorenal spaces were found to be more favorable for brephoplastic grafts than the subcutis.

(7) Larger cysts were formed when embryonic intestinal tissues were transplanted intramuscularly in tumor-bearing hosts than in normal hosts. However, the cysts showed no evidence of malignant invasion to the adjacent tissues.

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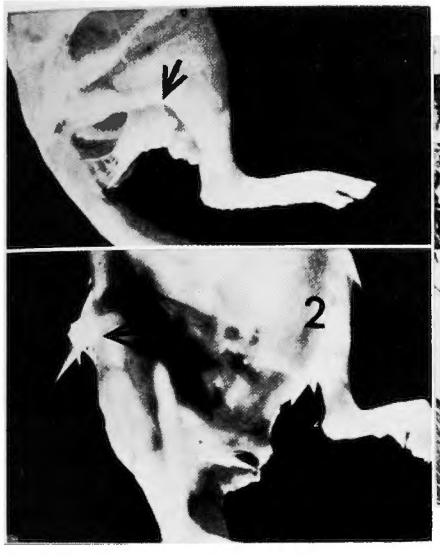
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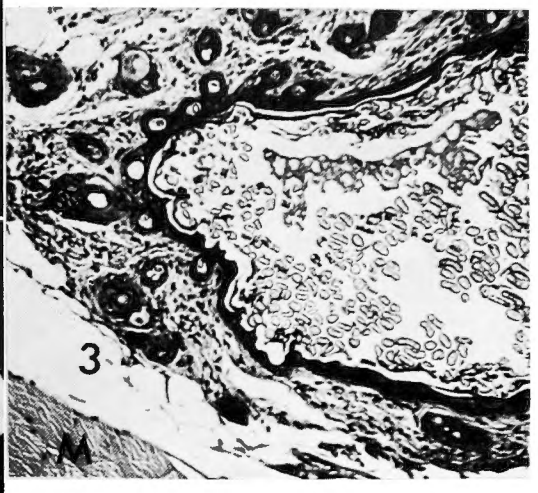
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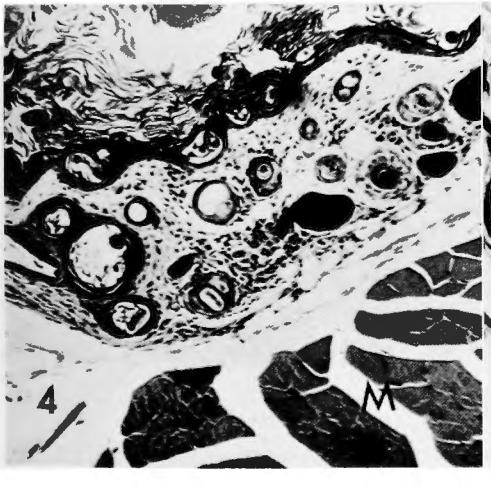
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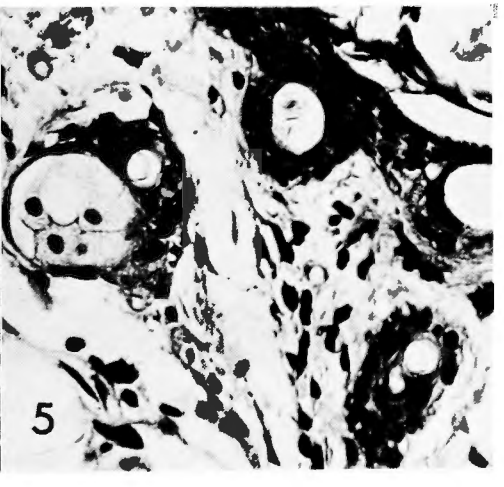
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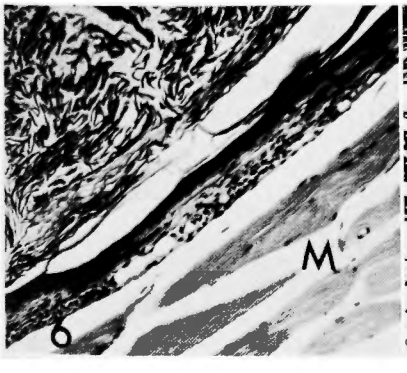
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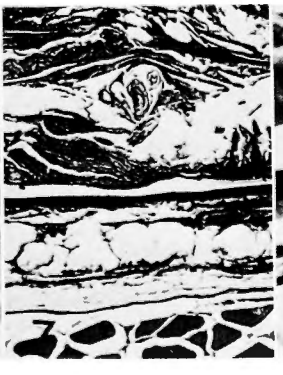
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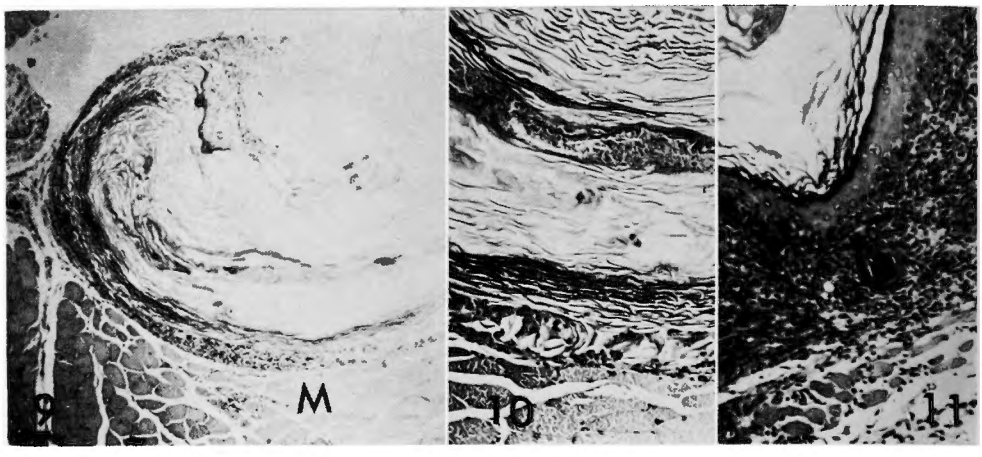
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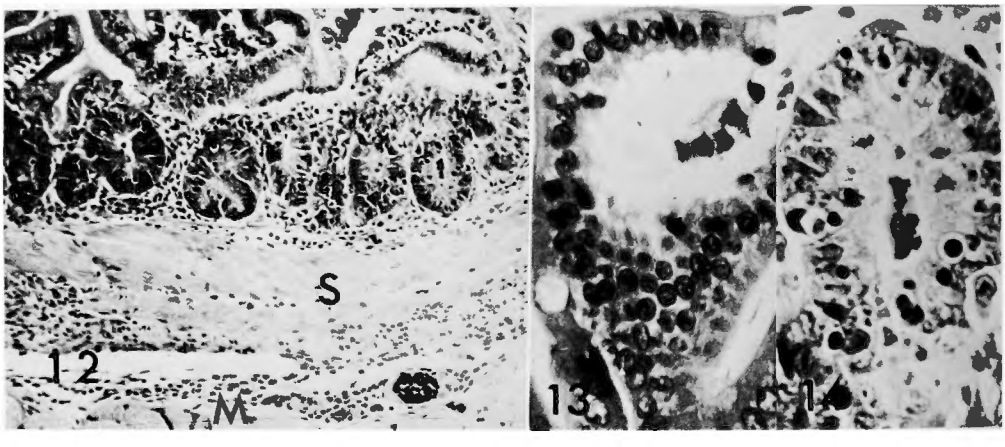
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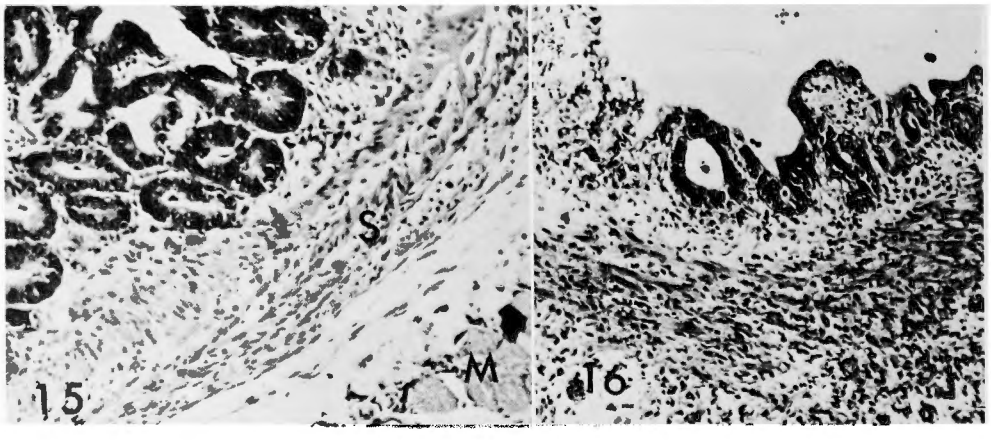
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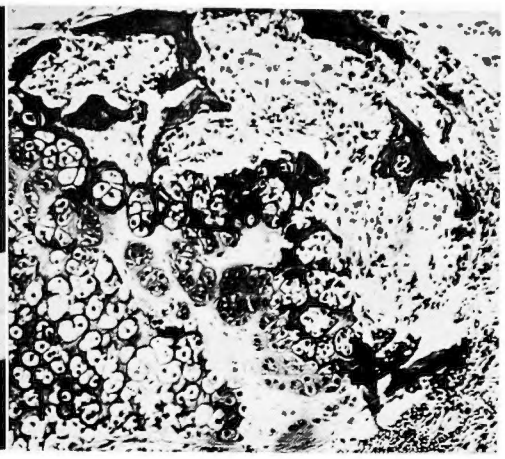

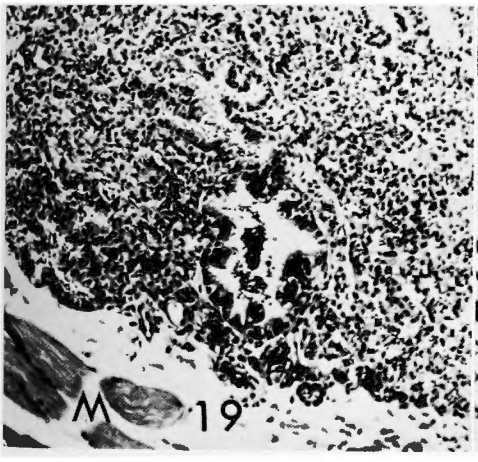
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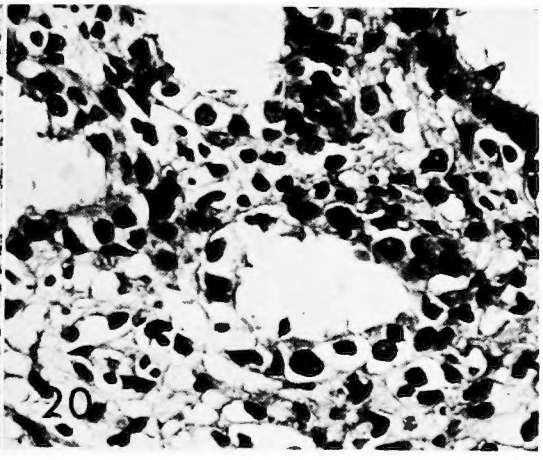
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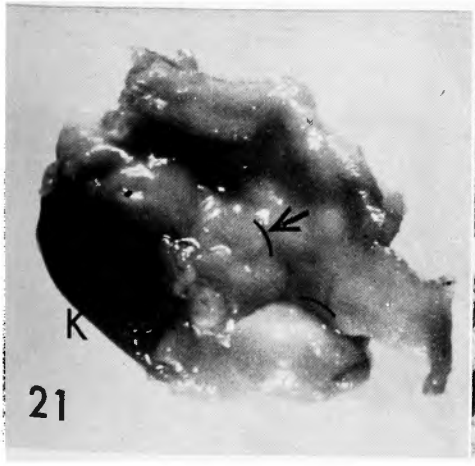
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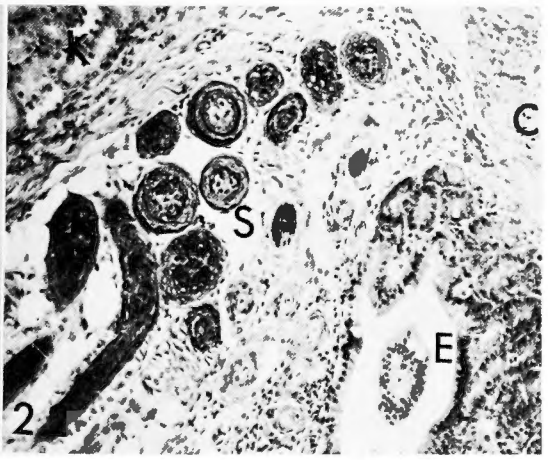
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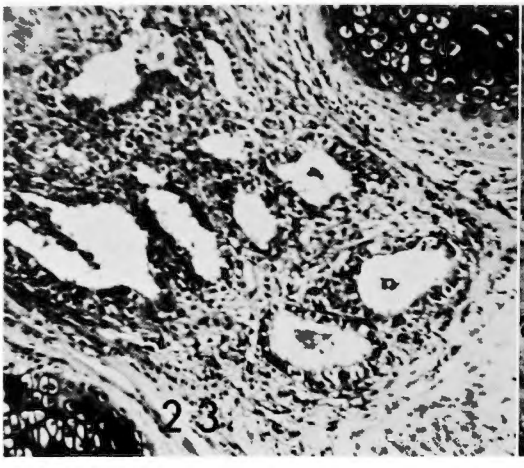

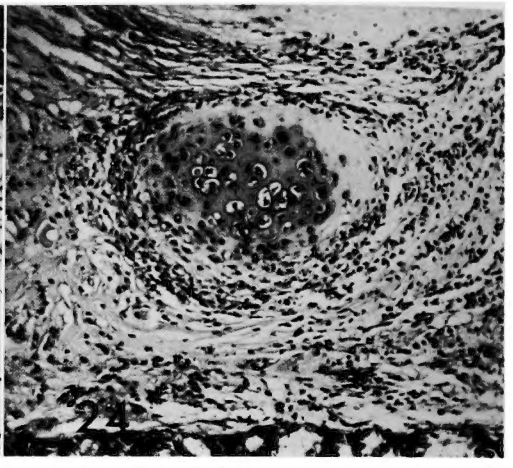

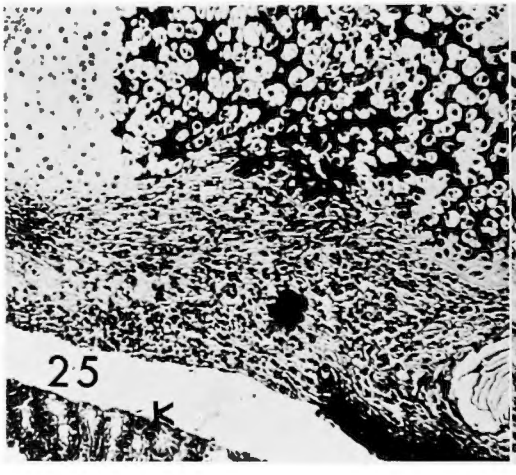

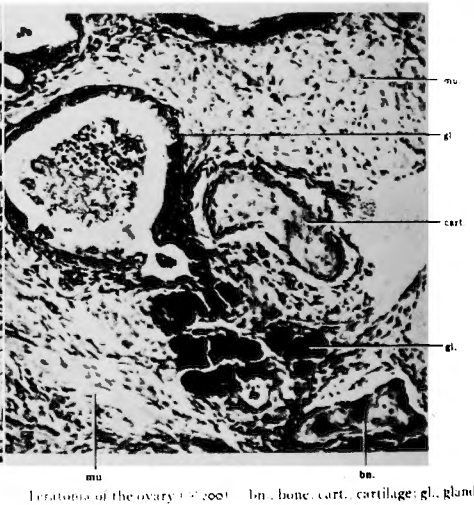

Teratoma of the ovary (×200). bn., bone; cart., cartilage; gl., gland; mu, striated muscle.

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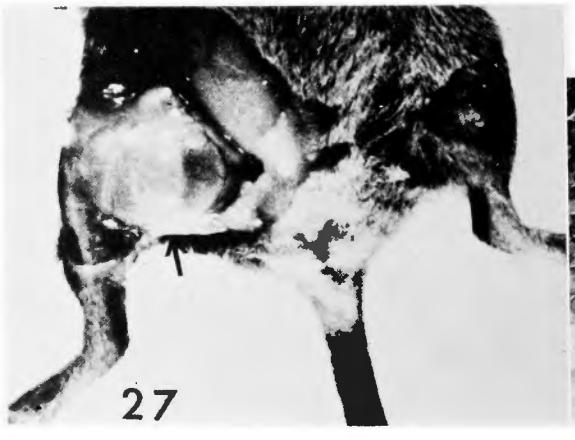

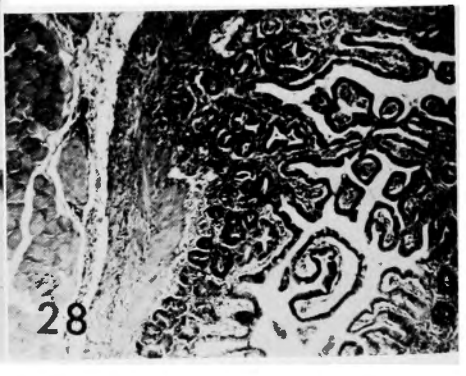

Explanation of Figures (2-28)

Fig. 2. Experimentally induced 'dermoid cyst' (60 days) was burst outward through the muscle sheets of host. Arrows point to actual projected hairs.

Fig. 3. 16-17 day embryonic skin graft, 45 days after isotransplantation into the thigh muscle. M: host's muscle. ×100.

Fig. 4. The same graft as Fig. 3, 67 days postoperatively. ×100.

Fig. 5. High power view of cyst wall shown in Fig. 4. Numerous hair follicles and sebaceous glands are healthy developing. ×400.

Fig. 6. Same graft, 276 days postoperatively. Cyst walls became thin, skin appendages disappeared. ×100. (all H. E.)

Fig. 7. The wall structure of 'epidermoid cyst'. ×100. (v. G.).

Fig. 8. High power view showing in Fig. 7. ×400. (v. G.).

Fig. 9. Intramuscular graft of 16-17 day embryonic skin, 21 days after homotransplantation (A/Jax-C3H). ×40.

Fig. 10. High power view of cyst wall showing in Fig 9. The developmental potential seems weak. ×100.

Fig. 11. Same homograft as Fig. 9, 28 days postoperatively. Intensive host reaction toward graft occurs. ×100.

Fig. 12. Intramuscular isograft of 16-17 day embryonic intestinal tissue, 28 days postoperatively. S: smooth muscle layers of graft, M: host's muscle. ×100.

Fig. 13. Mucous epithelium of same graft, 53 days postoperatively. Goblet clls are present. ×400.

Fig. 14. Same view, 121 days postoperatively. ×400.

Fig. 15. Intramuscular homograft of 16-17 day embryonic intestinal tissue, 21 days postoperatively (A/Jax-C3H). Normal arragements are still well kept. ×100.

Fig. 16. Homograft of the same kind as Fig. 15, 28 days postoperatively. Host reaction occurred increasingly and structures of graft were more or less destroyed. ×100. (all H. E.)

Fig. 17. Encystment at thigh indicated by arrow, 53 days after embryonic intestinal tissue was transplanted isologously.

Fig. 18. Bone marrow formation with ostoblasts and trabeculae, 21 days after minced whole tissue were homotransplanted to the muscle. ×100.

Fig. 19. Intramuscular isograft of 16-17 day embryonic lung, 28 days postoperatively. Most lung tissues collapsed. ×100.

Fig. 20. High power view showing in Fig. 19. ×400.

Fig. 21. 'Teratoma' induced in the retrorenal space (arrow), 180 days after 14-15 day embryonic minced whole tissues were transplanted. K: host's kidney.

Fig. 22. Minced whole tissues, 21 days postoperatively. Skin organs (S), cartilages (C) or mucous epithelium (E) reveal in the same section. K: host's kidney. ×100. (all H. E.)

Fig. 23. Another view of same graft as Fig. 22, 28 days postoperatively. In this section, cartilages, respiratory epithelium or smooth muscle like tissues are present. ×100.

Fig. 24. Same graft as Fig. 23, 120 days after isotransplantation. ×100.

Fig. 25. Minced whole tissues, 21 days after homotransplantation (A/Jax-C3H). ×100.

Fig. 26. Histology of spontaneously arosed teratoma of ovary in a mouse (from 'Biology of the Laboratory Mouse[34]'). Compare with Figs. 22, 23, 24 and 25.

Fig. 27. Huge cyst induced from 16-17 day embryonic intestinal tissue which was transplanted to tumor-bearing host, 35 days postoperatively.

Fig. 28. Histological appearance of this cyst. Infiltrative invasion is absent. ×40. (all H. E.)

和文抄録

胎児組織移植の実験的研究

京都大学医学部外科第2講座（指導：木村忠司教授）

原　　慶　文

胎令後期（14乃至15日以後）のマウス胎仔の種々組織を免疫学的成熟に達している平均2ヵ月の成熟マウスの数種の部位に同系並びに同種性異所性に移植し，その移植片の示す生物学的特徴を観察した．そして，胎児組織の同種移植抗原性の発現時期の問題，胎児組織移植の臨床的応用の可能性の問題を究明せんとした．更に，未分化の細胞構成を有する胎児組織細胞が異所性に迷入した時，その迷入片が如何なる運命を辿るかを長期に亘つて組織学的観察を続けた．これは迷入組織の悪性化という問題を解明する端緒ともなり得ると考えられる．以上の実験の結果は次の如くである．

(1) 一般に，胎仔の皮膚，胃腸，軟骨及び肺組織は同系マウスの大腿筋層内で高率に生着した．平面的構成をなして移植された皮膚片は，2乃至3週目で，立体的構造を有する嚢胞を形成した．組織学的には，"dermoid cyst" であつたが，やがて嚢胞壁の皮膚附属器官が消失して，"epidermoid cyst" の像を呈するに到つた．胃腸組織片もよく生着し，内部に液体を有するcyst を形成し，その壁は成体の胃腸と同一の配列構成を呈していた．

(2) 同種移植に於ても，皮膚・胃腸組織は同系のそれと同様 cyst を形成して生着したが，時間の経過と共に宿主側の細胞浸潤が強度となつたため5週以内に全例 reject された．

(3) 皮膚・腸組織を一定の移植片とし，成熟マウスの大腿筋層内を一定の部位とし同所性に同系並びに同種移植を試み，肉眼的組織学的に両者間の take の差を比較した．この実験結果から，マウスの胎令後期の該組織には既に同種移植抗原 antigenic specificity が相当量出現していることが推測される．

(4) いわゆるミンチにした胎仔全組織"minced whole tissues" を腎後部へ移植すると，円形の腫瘤に成育した．この腫瘤は組織学的には3胚葉性起原の組織が混然と発育しており "mixed tumor" もしくは "teratoma" の像であつた．しかし，同系で238日の観察期間中いずれの組織細胞も正常の発育過程を辿り，宿主側へ積極的に浸潤していくが如き異常を認めなかつた．

(5) 内胚葉性起原の心・肝・腎組織は同系同種共いずれの部位においても生着せず，吸収壊死に陥つた．

(6) 移植部位の良否の点からみる時，brephoplastic graft にとつて，大腿筋層内，腎後部は皮下部位よりも favorable であつた．特に前者は手技の簡単な部位であり，brephoplastic homograft の生着期間がやや延長する傾向がある点，臨床面への応用に希望を抱かせる．

(7) 担癌という異常性が brephoplastic graft に何らかの相関々係を有していないかどうかを調べた．担癌マウスの筋内へ胎令16乃至17日の同系胎仔腸組織片を移植すると，この群において最も大きな cyst を形成した．しかし，嚢腫壁から近接組織への異常浸潤の像が認められない．又，担癌動物が brephoplastic homograft の生着を促進する傾向を認め得なかつた．